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PATENT
0147-0189P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	Rainer FISCHER et al.	Conf.:	1798
Appl. No.:	09/419,788	Group:	1644
Filed:	October 18, 1999	Examiner:	M. E. Jamroz
For:	MOLECULAR PATHOGENICIDE MEDIATED PLANT DISEASE RESISTANCE		

DECLARATION UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, Dr. Paul Christou do hereby declare the following.

I have been asked to give my expert opinion regarding the above-captioned patent application.

Attached hereto is a copy of my *curriculum vitae*, which supports that I am fully knowledgeable of the field of the present invention.

I have been specifically asked to offer my opinion as to what invention I believed the inventors to be in possession of based on the description provided in the patent application specification.

In preparing my opinion, I reviewed the specification of the present patent application, including the pending claims. I further reviewed the rejection issued by the Examiner. In reaching my opinion, I relied on not only the information contained in the specification, but my general knowledge in the fields of plant molecular biology and protein biochemistry, as

well as readily available resources such as published resources, which were available as of 1998.

Having read the specification, I believe that the invention provides fusion proteins having a binding domain, for example, an antibody, and a membrane localization domain that leads to membrane anchoring. It is my understanding from the specification and from what is commonly known about membrane localization domains, that the presence of the membrane localization sequences permit the display of the binding domain on the lumenal face of organelles of secretory or endocytic pathways or plant cell membranes. Thus, a significant advantage of the fusion proteins of the invention is that the constructs can be targeted to the intracellular space where many pathogens are most vulnerable. In addition, as described on page 7, lines 23-25 of the specification, membrane localization of a binding domain stabilizes the protein and reduces C-terminal degradation *in vivo*. In my opinion, the finding that transgenic plants expressing a fusion protein of the invention have improved resistance against TMV infection was unexpected.

It is readily apparent to me upon reading the specification that the gist of the present invention is to anchor the binding domain of a fusion protein to the membrane of the plant cell and to thereby protect the plant from the pathogenic infection.

In my opinion, once the general concept of the invention, that being to anchor the binding domain of a fusion protein to

the membrane of the plant cell to thereby protect the plant from the pathogenic infection, was disclosed by the inventors, the general principal would be readily adapted to any desired plant pathogen or plant compartment. It falls within the skill of the average plant biologist to provide an antibody against a desired plant pathogen and to then combine it with a desired membrane localization sequence to target the construct to the desired plant cell compartment. Attached hereto is a copy of several documents, which support my opinion.

It was readily apparent to me upon reading the specification, that the specific examples in the specification only serve to illustrate the principle underlying the invention and that this principle is generally applicable to any desired plant pathogen by using a corresponding antibody domain in connection with a suitable membrane targeting sequence. In my opinion, the specification sufficiently describes the above-described principle that I considered the inventors to be in possession of it when the specification was written.

I each hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may

jeopardize the validity of the application or any patent issued thereon.

July 25, 2002
Date

PAUL CHRISTEN

D1. Chen, Y. D. & Chen, T. A. (1998) Expression of engineered antibodies in plants: A possible tool for spiroplasma and phytoplasma disease control. Phytopathology 88,1367-1371.

D2. Fecker, L. F., Koenig, R. & Obermeier, C. (1997) Nicotiana benthamiana plants expressing beet necrotic yellow vein virus (BNYVV) coat protein-specific scFv are partially protected against the establishment of the virus in the early stages of infection and its pathogenic effects in the late stages of infection, Arch Virol. 142, 1857-63.

D3. Le Gall, F., Bove, J. M. & Garnier, M. (1998) Engineering of a single-chain variable-fragment (scFv) antibody specific for the stolbur phytoplasma (Mollicute) and its expression in Escherichia coli and tobacco plants., Appl Environ Micro. 64, 4566-4572.

D4. E. E. Hiatt, N. S. Hill, & E. N. Hiatt (2001) Monoclonal antibodies incorporated into Neotyphodium coenophialum fungal cultures: inhibition of fungal growth and stability of antibodies. Fungal Genetics and Biology 33, 107-114.

D5. Schillberg, S., Zimmermann, S., Findlay, K. & Fischer, R. (2000) Plasma membrane display of anti-viral single chain Fv fragments confers resistance to tobacco mosaic virus, Molecular Breeding. 6, 317-326.

D6. Schillberg, S., Zimmermann, S., Zhang, M. Y. & Fischer, R. (2001) Antibody-based resistance to pathogens, Transgenic Research. 10, 1-12.

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D8. Vine ND, Drake P, Hiatt A & Ma JK. (2001) Assembly and plasma membrane targeting of recombinant immunoglobulin chains in plants with a murine immunoglobulin transmembrane sequence. Plant Mol Biol 45, 159-67.

D9. Voss, A., Niersbach, M., Hain, R., Hirsch, H. J., Liao, Y. C., Kreuzaler, F. & Fischer, R. (1995) Reduced virus infectivity in N. tabacum secreting a TMV-specific full-size antibody., Molecular breeding. 1, 39-50.

D10. Xiao, X. W., Chu, P. W. G., Frenkel, M. J., Tabe, L. M., Shukla, D. D., Hanna, P. J., Higgins, T. J. V., Müller, W. J. & Ward, C. W. (2000) Antibody-mediated improved resistance to CIYVV and PVY infections in transgenic tobacco plants expressing a single-chain variable region antibody, Molecular Breeding. 6, 421-431.

D11. Zimmermann, S., Schillberg, S., Liao, Y. C. & Fischer, R. 1998) Intracellular expression of TMV-specific single-chain Fv fragments leads to improved virus resistance in Nicotiana tabacum., Molecular Breeding. 4, 369-379.

CURRICULUM VITAE

NAME:	Paul Christou
DATE OF BIRTH:	July 5, 1954
CITIZENSHIP:	USA
DEGREES:	BSc. 1st Class Honours (1977) University of London, Chelsea College, United Kingdom Organic and natural products chemistry
	PhD (1980) University of London, University College, United Kingdom Biosynthesis of secondary products in plant cell cultures. Plant Biochemistry.

POSITIONS HELD

2001-present	Fraunhofer Institute of Molecular Biotechnology and Applied Ecology. Schmallenberg-Aachen, Germany
2000 - present	Appointed to the Scientific Advisory Board of the Center for Research and Technology Hellas (CERTH), Ministry of Development, General Secretariat of Research and Technology, Greece (June 2000).
1998 - 2001	John Innes Centre, UK Head, Molecular Biotechnology Unit Band 3, Rank of Full Professor
1994 - 1998	John Innes Centre, UK Head, Laboratory of Transgenic Technology & Metabolic Pathway Engineering Band 4, Rank of Associate Professor
1997 - present	Full Adjunct Professor Mediterranean Agronomic Institute Chania, Crete, Greece
1994 - 1995	European Commission. Chairman of Biotechnology Bridge Programme evaluation panel.
1993 - 1997	ABSP Technical Advisory Board Panel Member Michigan State University, USA
1988 - 1994	Agracetus Inc. USA Senior Scientist Molecular and Cellular Plant Biology Secondary products Medicinal and aromatic plants Legume and cereal crop transformation

1982 - 1987	Agracetus. Scientist; Project Leader Plant Cell & molecular biology; organic chemistry
1980 - 1982	University College London Research Fellow - Junior Lecturer Natural products chemistry; biosynthesis of natural products

PROFESSIONAL SOCIETY MEMBERSHIPS

American Tissue Culture Association
International Society of Plant Molecular Biology
International Tissue Culture Association
Royal Society of Chemistry (UK)
American Chemical Society
American Association for the Advancement of Science
Member of European Leguminous Crops Working Group
The Biochemical Society (1998)

RESEARCH INTERESTS

Our programmes focus on three major activities in three distinct areas. These are: Transgene structure and function in genetically engineered crop species; metabolic engineering of secondary product pathways in medicinal plants; production of pharmaceutical macromolecules in crop plants. Our laboratory also has a mandate from the Rockefeller Foundation to act as a training and technology transfer centre for rice and tropical maize biotechnology. These activities are discussed in more detail below.

I. Transgene structure and function in genetically engineered crops

Our activities in this area are supported by the Rockefeller Foundation, the DFID (UK, Department for International Development), the BBSRC (UK National programme funding biotechnology research), the EU and commercial sponsors. We have projects on rice, tropical maize, sugarbeet, wheat, pea, tomato and pigeonpea. This programme involves generation of transgenic plants and a detailed study of molecular parameters which influence transgene stability and expression. We have 15 collaborators globally and our transgenic rice has been undergoing field trials in two locations (Italy and USA) for the past several years. The objectives of our activities are both basic and applied. In addition to answering fundamental molecular biology questions, we also have programmes targeting introduction and study of agronomically-useful genes into all our target species. These include genes for insect resistance (Bt, protease inhibitors and lectins, for rice and maize), virus (covering South and Central America, East Africa and South and SE Asia, for rice), nematode resistance (West African rice), dough quality and stability (wheat) etc. Additional activities focus on genomics research involving transposon mutagenesis, introduction of multiple genes into plants and detailed gene expression studies. Projects in this programme are fully integrated both horizontally and vertically, within the Institute, the UK, the EU and globally.

II. Metabolic engineering of secondary product pathways in medicinal plants

This activity is supported by the JIC, commercial sponsors, EMBO, The von Humboldt Institute of Colombia and the Colombian government. We are studying the effect of manipulating genes involved in indole and tropane alkaloid metabolism in two pairs of related species, Catharanthus roseus and Lonicera tartarica and Nicotiana and Hyoscyamus. The aim of the project is to generate novel chemotaxonomic entities by manipulating enzymes of secondary metabolism in these plants. Our results strongly indicate that secondary metabolism is indeed very plastic and LC-MS analysis has revealed the appearance of novel compounds in experiments in which introduced genes are targeted to specific sub-cellular compartments. We have a

collaborative relationship in place with a pharmaceutical company and in the framework of this relationship we have gained access to sophisticated screening technologies allowing us to screen extracts from transgenic plants for novel pharmacological activity. Major objectives of the project include the creation of hybrid secondary products with novel biological activity, utilization of engineered plants and cell cultures in bio-transformation experiments to create novel substitution patterns, unravelling the mechanisms of secondary metabolic pathway flux control and the creation of alternative crops for non-food applications.

III. Production of pharmaceutical macromolecules in crops

We have long term collaborations with colleagues at Guy's Hospital and the University of Aachen, Germany, utilizing wheat, rice and pea as production vehicles for important pharmaceutical macromolecules such as antibodies and vaccines. Specific targets have been carefully selected to assure that there will be no competition from transgenic microorganisms, mammalian cell cultures and transgenic animals. We have demonstrated proof of concept in terms of the feasibility of using plants as production vehicles for such products and we are now engaged in activities to ascertain the safety of such macromolecules produced in transgenic crops. In the context of this project we are studying protein sorting and trafficking and these studies have shed more light on fundamental biochemical processes which take place in different compartments in the plant cell.

IV. Training and technology transfer centre

Training and technology transfer activities in our laboratory are funded by the Rockefeller Foundation and focus on training and technology transfer to developing country scientists working on rice and tropical maize biotechnology. Our laboratory is one of the only two laboratories in the western world funded to carry out this function. The training programme includes all aspects of molecular and cellular biology, including construct making, gene transfer, molecular and biochemical analysis as well as field trials. Under this umbrella we are also involved in intellectual property issues, regulations governing release of transgenic plants and public perception.

ADMINISTRATIVE EXPERIENCE

Co-Director of the Fraunhofer Institute of Molecular Biotechnology and Applied Ecology, Schmallenberg, Germany. Leading a new Institute with emphasis on applied aspects of plant biotechnology and novel uses of transgenic plants.

Head of the Department of Molecular Biotechnology at the John Innes Centre, Norwich, UK. The JIC is one of the premier centres in Europe for Plant Biomolecular Sciences (over 900 employees). Direct responsibility for management and administration of 45 individuals encompassing research and administration. Fund raising activities to sustain these individuals. Current level of funding is in excess of UK£ 1,2 million per annum. I have put together this department from ground zero since I joined the JIC in 1994. Member of JIC Central Management Committee, the remit of which is to administer resource allocation at the entire Centre. Member of the JIC Central Scientific Strategy Committee, which is responsible for setting and implementing science policy at the JIC and liaising with the Biology and Biotechnology Science Research Council of the UK, which is the government body responsible for running UK-Research Institutes.

PUBLICATIONS

BOOKS

1. Christou, P. (1996) (Author) Gene Transfer into Plants by Particle Bombardment. R.G. Landes Company, Austin, Texas.

2. Yang, N-S. and Christou, P. (1994) (Editors) Particle Bombardment Technology for Gene Transfer. W.H. Freeman and Co., Publishers, New York, N.Y., USA. p.202

3. Christou, P. (1994) (Author) Rice Genetic Engineering. Technomic Publishing Co, Inc. Lancaster PA, USA. p. 211

4. Christou, P. (1993) (Author) Genetic Engineering and in Vitro Culture of Crop Legumes. Book published by Technomic Publishing Co, Inc. Lancaster PA, USA. p. 307

CHAPTERS IN BOOKS AND CONFERENCE PROCEEDINGS

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18. Eva Stöger, Yolande Perrin, Liz Nicholson, Esperanza Torres, Richard M. Twyman, Samantha J. Bean, Carmen Vaquero, Rainer Fischer and Paul Christou. Production of recombinant diagnostic and therapeutic antibodies in transgenic cereals and legumes. Methods in Molecular Biology The Humana Press Inc. In Press.

17. Leech, M., Burtin, D., Hallard, D., Hilliou, F., Kemp, B., Palacios, N., Rocha, P., O'Callaghan, D., Verpoorte, R., Christou. P. Particle gun methodology as a tool in metabolic engineering. In: Metabolic engineering of plant secondary metabolism. Eds. R.Verpoorte and A.W. Alfermann. Landes Bioscience, Texas, USA (in press).

16. Pascal Drake, Eva Stoger, Liz Nicholson, Paul Christou and Julian K. C. Ma. Antibody Production in Plants. In: "Monoclonal Antibodies - A Practical Approach". Edited by P. Shepherd and C. Dean. Published by Oxford University Press, 2000.

15. I Kurek, R Dulberger, P. Christou, A Breiman (1999). Studies on wheat prolyl isomerase in transgenic plants. In: Plant Biotechnology and In Vitro Biology in the 21st Century - Proceedings of the IXth International Congress of the International Association of Plant Tissue Culture and Biotechnology, Jerusalem, Israel, 14 - 19 June 1998. Edited by A Altman, M Ziv (Faculty of Agriculture, Rehovot, The Hebrew University of Jerusalem, Israel) and S Izhar (Agricultural Research Organization, Bet Dagan, The Ministry of Agriculture, Israel). Published by Kluwer Academic Publishers.

14. F Sala, A Arencibia, S Castiglione, P Christou, Y Zheng, Y Han (1999). Molecular and field analysis of somaclonal variation in transgenic plants. In: Plant Biotechnology and In Vitro Biology in the 21st Century - Proceedings of the IXth International Congress of the International Association of Plant Tissue Culture and Biotechnology, Jerusalem, Israel, 14 - 19 June 1998. Edited by A Altman, M Ziv (Faculty of Agriculture, Rehovot, The Hebrew University of Jerusalem, Israel) and S Izhar (Agricultural Research Organization, Bet Dagan, The Ministry of Agriculture, Israel). Published by Kluwer Academic Publishers.

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11. Christou P. (1998) Book Chapter: Cereals. In: Genetic Modification in the Food Industry: A strategy for food quality improvement. Edited by Sibel Roller and Susan Harlander. Published by Blackie Academic & Professional.

10. Christou P. (1997) Maize Biotechnology: Recent Developments in Maize Transformation and Applications. The Proceedings of the XVIIth Conference on Genetics, Biotechnology and Breeding of Maize and Sorghum held at the Aristotelian University of Thessaloniki, Greece on October 20 - 25, 1996. A. Tsaftaris, Edt., Royal Soc. Chemistry. pp. 79-87.

9. Christou, P., P. Vain, A. Kohli, M. Leech, J. Oard and S. Linscombe (1996). Evaluation of transgene stability, gene expression, and field performance of herbicide-resistant transgenic plants. Proceedings of the 3rd International Rice Genetics Symposium, Manila, The Philippines. October 16-20, 1995.

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5. Christou, P. (1995). Particle Bombardment. Methods in Cell Biology 50: 375-382. Academic Press, Inc.

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3. Christou, P. (1994). Particle Bombardment-Application to plants. In: Particle Bombardment Technology for Gene Transfer. Oxford University Press, New York, N.Y., USA.. Yang and Christou Edts. 71-99.

2. Christou, P., McCabe, D.E., Swain W.F., and Russell D.D. (1993) Legume transformation. In Control of Plant Gene Expression (D.P.S. Verma, ed.) Telford Press, Caldwell, New Jersey pp.547-564

1. Christou, P. (1991) Particle bombardment-mediated transformation of organized tissue and its impact on agricultural biotechnology. Intl. Assoc. Plant Tissue Culture. 66:2-14

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109. E. Stoger, M. Sack, Y. Perrin, Vaquero, E. Torres, R. Twyman, P. Christou and R. Fischer. Practical considerations for pharmaceutical antibody production in different crop systems. Mol Breed. In press

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101. O. Lepri, L. Bassie, G. Safwat, Pham T.-H, Pham T.-N., E. Hölttä, P. Christou and T. Capell Over-expression of the human *ornithine decarboxylase* cDNA in transgenic rice plants alters the polyamine pool in a tissue-specific manner. Mol Gen Genet. 266: 303-312.

100. X. Fu, D. Sudhakar, J. Peng, D. E. Richards, Paul Christou and N. P. Harberd (2001). Expression of *Arabidopsis* GAI in transgenic rice represses multiple rice gibberellin responses The Plant Cell 13: 1791-1802.

99. S. Elumalai, L. Bassie, P. Christou and T. Capell (2001). Development of a novel gene transfer system for pigeonpea [*Cajanus cajan* (L.) Millsp.] and expression of a monocot *arginine decarboxylase* cDNA in transformed cell lines. Plant Physiol Biochem. 39: 575-582

98. Ajay Kohli, Jianhua Xiong, Raffaella Greco, Paul Christou and Andy Pereira (2001). Tagged Transcriptome Display (TTD) in *Indica* Rice using *Ac* Transposition. Mol. Gen Genet. 266: 1-11

97. K. Tang, X. Sun, Q. Hu, A. Wu, C. Lin, H. Lin, R.M. Twyman, P. Christou, T. Feng. (2001). Transgenic rice plants expressing the ferredoxin-like protein (AP1) from sweet pepper show enhanced resistance to Xanthomonas oryzae, pv. Oryzae. Plant Science 160: 1035-1042

96. Eva Stoger, Mary Parker, Paul Christou and Rod Casey (2001). Pea legumin overexpressed in wheat

endosperm assembles into an ordered paracrystalline matrix. Plant Physiol. 125: 1732-1742

95. R. Greco, P.B.F. Ouwerkerk, C. Sallaud, A. Kohli, L. Colombo, P. Puigdomènech, E. Guiderdoni, P. Christou, J.H.C. Hoge, and A. Pereira (2001). Transposon Insertional Mutagenesis in Rice. Plant Physiol. 125: 1175-177.

94. Shahina Bano Maqbool, Sheikh Riazuddin , Nguyen Thi Loc,, Angharad M. R. Gatehouse, John A. Gatehouse, & Paul Christou. (2001) Expression of multiple insecticidal genes confers broad resistance against a range of different rice pests. Mol. Breeding 7: 85-93

93. Luke Mehlo, Gatsha Mazithulela, Richard M. Twyman, Margaret I. Boulton, Jeffrey Davies and Paul Christou (2000) Structural analysis of transgene rearrangements and effects on expression in transgenic maize plants generated by particle bombardment. Maydica 45: 277-287 .

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ISSUED PATENTS

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Particle-mediated transformation of soybean plants and lines
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Particle-mediated transformation of soybean plants and lines
European Patent No. 88306613.6
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Method of creating a transformed rice plant
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Plant Transformation process with early identification of germline transformation events
European Patent Application No: 0444882A2. Date: April, 9, 1991

5. Christou, P and McCabe, D.
Plant Transformation process with early identification of germline transformation events
US Patent Number 5,503,998.
April 2, 1996.

PENDING PATENTS

1. Fatima Pelica and Paul Christou. Sugarbeet transformation method.

2. Luke Mehlo and Paul Christou. Novel insecticidal fusion proteins.

3. Eva Stöger, Rainer Fischer and Paul Christou. Expression of therapeutic and diagnostic antibodies in cereals species.

4. Ajay Kohli and Paul Christou. Genetic transformation using clean DNA fragments.

5. Teresa Capell and Paul Christou. Polyamine accumulation in cereal grains.

6. Teresa Capell and Paul Christou. Methods for Reducing Polyamine Biosynthesis and the Plants Produced Thereby.

7. Frederique Hilliou, Paul Christou and Mark Leech. A novel peroxidase that catalyses the production of the anticancer agents vinblastine and vincristine from the medicinal plant, Catharanthus roseus.

8. P. Christou and J.C. Cooley. Process for enhancing or modifying levels of medicinal secondary products in transgenic plants.

9. P. Christou. A novel expression system for the cloning of genes important in secondary product biosynthesis.

10. P. Christou, D.E. McCabe and D.R. Russell. Legume transformation.

11. P. Christou, T. Ford and M. Kofron. Genotype-independent transformation of rice.

12. P. Christou and T. Ford. Variety-independent transformation method for maize, wheat and barley from dry seed.

13. P. Christou. Novel method for genetic engineering of elite maize varieties.

14. G. Brar, D. McCabe and P. Christou. Genetic transformation in peanut.

Other Activities:

Panel Member - evaluation for Greek Ministry of Science and Technology (1999).

External examiner for PhD candidate at the Institute for Biological and Experimental Technology, (IBET), Oeiras, Portugal (January 15, 1999).

External examiner for PhD candidate at Leiden/Amsterdam Center for Drug Research, Leiden University, Leiden, Netherlands (April 14, 1999).

Organizing Committee and Session Chairman, International Association for Plant Tissue Culture, IX International Congress on Plant Tissue and Cell Culture, Jerusalem, Israel (June 14-19, 1998)

Member of Advisory Committee. Biotechnology in the food chain: New tools and applications for Future Foods. International Symposium, organised by VTT Biotechnology and Food Research, Helsinki, Finland, in collaboration with ATO, Netherlands. Helsinki, Finland, (28-30 January 1998)

Organizing Commitee for EURESCO Congress, Helsinki, Finland (September 1997)

External examiner for PhD candidate at De Montfort University (1997).

Graduate course organizer at Institute of Molecular Biology and Biotechnology and University of Crete (1997)

Co-organizer of EU-sponsored workshop on use of plants for alternative uses, including vaccines, antibodies and secondary products (Institute of Food Research, Norwich, UK; June 1996).

Organizer of EU-Funded Secondary Product Engineering Workshop (John Innes Centre, Norwich, UK; April 15-16, 1996)

Position paper on Molecular Farming and Secondary Product Engineering for FAIR (1996).

Position paper on Secondary Product Engineering for BIOTECH (1996).

EDITORIAL WORK

1. Plant Journal - Editorial Advisory Board (1990-1996)
2. Plant Science - Editorial Advisory Board (1994-1998)
3. Molecular Breeding - Editorial Board (November 1998 -)
   Acting Chief Editor (April 2002-to date)
4. Transgenic Research - Editorial Board (1998 -1999)
5. Transgenic Research - Chief Editor (1999 – to date)
6. IAPTC&B - Editorial Board (2000 – to date)